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(54) Title: PROCESS FOR ENRICHING THE PROTEIN CONTENT OF FOOD AND FEEDSTUFF AND PRODUCTS THEREOF

(57) Abstract

A method for producing a protein enriched food or feed product and products thereof. The method entails forming a mixture of water and a food or feed product, sterilizing the mixture, inoculating the sterilized mixture with a spawn culture of the genus *Pleurotus*, maintaining the inoculated mixture in the presence of air at a temperature of from about 5 to about 46°C so as to enable the mycelium of the spawn culture to grow, and later terminating the growth of the mycelium. The food or feed product resulting from this process has an increased protein content.

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PROCESS FOR ENRICHING THE PROTEIN CONTENT OF FOOD AND FEEDSTUFF AND PRODUCTS THEREOF

BACKGROUND OF THE INVENTION

This invention relates generally to food or feed products and processes. More particularly, this invention relates to a process for enhancing the protein content of food or feed products. Additionally the invention relates to food and feed products having an increased protein content as produced by the subject process.

One of the most serious problems which the world faces is supplying nutritionally adequate protein to meet the demands of a population which is expanding by approximately 55 million people annually. If the average protein requirement for an individual is about 52 pounds per year, this means that there must be produced an additional 2.8 billion pounds of protein each year just to keep pace with the population increase. This does not take into account the fact that much of the present world population does not have sufficient protein in their diet to be healthy enough to lead meaningful lives. Thus, there is a very pressing need for ways of increasing the total protein production of the world.

A problem associated with the need to supply adequate protein for the world population is the fact that cereal grains and cereal grain products make up a high percentage of the diet of most of the developing nations. Unfortunately,

these grains and grain products usually contain 10% or less protein which is very often deficient in some of the essential amino acids. Since the average person requires at least 65 grams of high quality protein per day for good health, this means that an intake of 650 grams of cereal grains would be required to supply this quantity of protein. Ingesting this much cereal grain would supply too much carbohydrate relative to the amount of protein. Furthermore, the protein contained within these cereal grains is generally of relatively poor quality.

The present invention is directed to a solution to the above-described problems by increasing the ratio of protein to carbohydrate in cereal grains and products thereof while concominantly improving the overall amino acid balance for the treated grain.

Much of the grain grown in the world is fed to monogastric animals (swine, chickens, etc.) who have roughly the same nutritional requirements as man. Accordingly, the present invention would lead to economy in feeding these animals since it would not be necessary to add protein supplements to these grains (soy bean meal, cotton seed meal, fish meal, etc.) if the animals were fed grains treated by the process of the present invention.

Studies have been made in the past to develop processes for treating various foods containing carbohydrates with lower fungi so as to increase their protein content. The process suggested in such studies, however, are less desirable than

the process of the present invention because these other processes either require "fixed" inorganic nitrogen, produce nutritionally deficient protein, or require separation of the protein to produce an acceptable food or feedstuff.

On the other hand, the present process suffers from none of these drawbacks. A food or foodstuff such as a cereal grain (wheat, corn, and the like) treated by the instant process may be dried to the customary moisture levels and processed by conventional methods to provide food and feed products such as bread, dry cereal products, dog and cat foods and the like which contain enough high quality protein to be nutritionally well balanced and have a flavor which is highly acceptable.

Another aspect of the present invention is the fact that agricultural waste products such as peanut hulls, corn residue, grain straws and the like may be mixed with cereal grains and treated by this process to give products which are particularly well suited for animal feedstuff. Materials such as corn silage could also be treated by the present process.

Additionally concepts have previously been disclosed relating to the art of cultivating mushrooms from a mycelium of the genus Pleurotus including Pleurotus ostreatus, Pleurotus ulmariums, Pleurotus sapidus, Pleurotus corinicopiae, and Pleurotus florida.

At least one publication suggests that mushrooms may be cultivated in a culture medium comprising, for example,

corn fibers and rice bran (or corn bran) and subsequently the cultivation medium may be used as feedstuff.

At least one other previously known publication discloses that a nutrient substance which comprises one or more cellulose-containing agricultural waste products may be crushed, mixed with water and placed into a ventilated container which is sterilized. The sterilization is carried out either by heating to 70 to 80°C for 5 hours or by introducing propylene oxide. After the mixture is placed inside the sterilized container, it is injected with a mycelium of the fungus Pleurotus ostreatus. The nutrient substance is preferably wheat, barley, rye, peas, rice shells, sun flower stalks and seeds.

The container is kept at a constant temperature between 16 and 20°C after seeding. When the nutrient is permeated with the mycelium, the temperature is lowered to between 5 and 16°C. After the onset of the crop of mushrooms, the container is opened and illuminated with light.

Still another theorist suggests that a substrate such as seed oil residues (e.g., cottonseed meal, coconut meal, peanut meal, etc.) which are advantageously mixed with a cereal material such as wheat or the like may be innoculated with a mycelium of mushrooms including, among others, Pleurotus ostreatus. The substrate (e.g., peanut meal) is first acid hydrolyzed by treating it with mineral acid. The calcium carbonate is added and the pH is adjusted to between

6 and 8. This mixture is then innoculated with the mycelium of mushrooms (e.g., Pleurotus ostreatus) which has been grown on a medium of the same composition.

Although the above noted disclosures have at least a degree of conceptual appeal in the art of growing mushrooms, little if any attention has been directed to developing a protein enriched food or food stuff using a spawn culture of the genus Pleurotus. In none of these publications has any mention been made of the fact that Pleurotus will grow in the presence of materials already containing protein (cereal grain and the like) without substantially altering the protein already present, and at the same time produce additional protein by fixing nitrogen which is in the air and using the grain as a source of carbon.

OBJECTS AND SUMMARY OF THE INVENTION

Accordingly, a general object of the present invention is to provide a novel process for preparing a food or feed product which has an enhanced protein content.

A more specific object of the present invention is to provide a novel process for increasing the protein content of food or feed products.

Another object of the invention is to provide a novel process for increasing the protein content of a food or food-stuff utilizing nitrogen from the atmosphere.

A further object of the invention is to provide a novel process for increasing the protein content of cereal grains while concomitantly improving the overall amino acid balance for the treated grain.

A still further object of the invention is to provide a novel process for increasing the protein content of agricultural waste products to produce a product particularly suited for animal feedstuff.

Still another object of the present invention is to provide a protein enriched food or feed product produced by this process.

Other objects and advantages of the invention will become apparent from the following summary and description of the preferred embodiments of the present invention.

Summary

In one aspect, the present invention provides a process for increasing the protein content of a food or feed product which contains carbohydrates.

This process comprises :

(a) forming a mixture of the food or feed product with water;

(b) sterilizing the mixture in order to substantially eliminate the growth of substances which would compete with a spawn culture of the genus Pleurotus;

(c) innoculating the mixture with a spawn culture of the genus Pleurotus;

(d) maintaining the innoculated mixture in the presence of air at a temperature of from

about 5 to about 46°C so as to enable the mycelium of the spawn culture to grow, and

(e) terminating the reproduction of the mycelium so as to form a food or feed product which has an increased protein content.

In another aspect, the present invention comprises the product produced by the above described process.

DESCRIPTION OF PREFERRED EMBODIMENTS

The process of the present invention involves treating a food or feed product with a spawn culture of the genus Pleurotus. The food products useful in this process include any carbohydrate containing food product on which Pleurotus mycelium will grow in the presence of air. Such products include, for example, cereal grains such as corn, rye, oats, milo, rice, barley, soybeans, manioc, yams, and sweet potatoes; products of the milling industry that contain carbohydrates such as wheat bran and rice bran; other carbohydrate containing food products such as lima beans, pinto beans, and pulses including white acre peas, black-eyed peas, and the like, and agricultural wastes such as peanut hulls, corn residue, grain straws, cottonseed hulls, bigasse, corn silage. Mixtures of two or more food or feed products may also be used.

The first step in the subject process comprises mixing the food or feed product with water. Water is needed so that when the food or feed product is innoculated with Pleurotus it will have a sufficiently moist environment in which to grow.

The mixture should contain generally from about

20% to about 80%, typically from about 30% to about 70% and preferably from about 40% to about 60% by weight food or feed product, and correspondingly generally from about 20% to from about 40% to about 60% by weight water. These percentages are based upon the total weight of the mixture.

In a preferred embodiment of the present invention, calcium carbonate may be added to the mixture of the food product and the water. The purpose of the calcium carbonate is to increase the pH of the mixture to a value in the range of from about 6 to about 8 for the purpose of providing the preferred pH for the growth of Pleurotus. The calcium carbonate is also added for the purpose of supplying nutrient to the mixture.

Although calcium carbonate is preferred, any compound which is non-toxic and which will supply required nutrients is useful in the growth of the Pleurotus mycelium. Such compounds include calcium hydroxide, calcium sulfate, dicalcium phosphate, organic calcium salts such as calcium citrate, and the like, and other mineral nutrient materials such as nutrient compounds of potassium, magnesium iron, phosphorus, zinc, copper, boron, sulfur, and the like. Mixtures of two or more nutrient compounds may also be used.

These nutrient compounds must be in a form such that they are not toxic to the mycelium. Harvested grains usually contain sufficient quantities of these nutrients and their addition is not required for efficient growth when harvested grains are employed.

The second step of the present process comprises sterilizing the mixture so as to substantially eliminate the growth of substances which might compete with the Pleurotus.

This mixture may be sterilized by any means known to those skilled in this art. For example, the mixture may be sterilized by heating or by pretreating the substrate with chemical compounds such as propylene oxide or anhydrous ammonia.

If the mixture is sterilized by means of heat, it should be heated to termperatures in excess of 120°C for a sufficiently long period of time to allow the entire mixture to attain a minimum temperature of 120°C.

The sterilized mixture is next innoculated with a spawn culture of the genus Pleurotus at temperatures which are generally from about 5°C to about 46°C typically from 10°C to about 30°C and preferably from about 20°C to about 30°C. Mixtures of two or more members of this genus may also be used.

As used herein, the term "spawn culture" refers to a living culture of the mycelium growing on any substrate which will support its growth.

The mixture may be innoculated with the spawn culture by adding a small amount of the culture as finely divided as possible and mixing this culture into the new substrate as thoroughly as possible. The amount of Pleurotus used to innoculate the food product mixture may be generally from about 0.5% to about 20%; typically from about 1% to about 5%, and preferably from about 1% to about 3% by weight based upon the total weight of the innoculated mixture.

The innoculated mixture is then maintained in the presence of a mixture of nitrogen and oxygen at a temperature

of generally from about 5°C to about 46°C, typically from about 10°C to about 35°C, and preferably from about 20°C to about 30°C. These temperature ranges are important in that they represent those temperatures at which Pleurotus will grow. At temperatures substantially below about 5°C or substantially above about 46°C Pleurotus does not experience substantial growth.

The innoculated mixture must be maintained in the presence of a mixture of nitrogen and oxygen in order to produce an increase in protein content of the food product.

Any ratio of nitrogen to oxygen may be employed in the process of the present invention although the mixture shall preferably contain more than 10% by weight oxygen and more than 10% by weight nitrogen. The amount of gas mixture which is employed is important only in that substantial protein increase may not occur if there is insufficient nitrogen and/or oxygen present during the growth period. Other gases may also be included in this mixture. Air which is at or near the surface of the earth is a preferred gas mixture since it contains oxygen and also contains approximately 78% molecular nitrogen by volume.

The innoculated mixture should be maintained in contact with the gas mixture for a period of time long enough to effect sufficient growth of the mycelium but not for too long a period of time since there is no advantage in doing so and since contamination with other fungi or bacteria may occur.

The innoculated mixture should be kept in contact with the gas mixture for generally from about 2 days to about 80 days, typically from about 14 days to about 56 days, and preferably from about 21 days to about 35 days.

If the innoculated mixture is kept in contact with the gas mixture for a time period substantially less than about 2 days, then the protein increase is comparatively insignificant. On the other hand, if the innoculated mixture is kept in contact with the gas mixture for a time period substantially in excess of 80 days, the amount of protein increase over and above that attained up until that time is also comparatively insignificant.

After sufficient mycelium growth has taken place, the growth of the mycelium is terminated. Growth may be terminated, for example, by dehydrating the mixture to a moisture content of generally less than about 20%, typically less than about 18%, and preferably less than about 12% by weight based upon the total weight of the innoculated mixture. Growth may also be terminated by chemical sterilization or heating.

Prior to treatment according to the process of the present invention, the food product has a protein content of generally less than about 25 percent, by weight, based upon the weight of the food product as determined by nitrogen analysis.

After treatment according to the process of the present invention, the food product has a protein content which is generally at least about 30 percent to 65 percent

by weight higher; based upon the weight of the food product.

The following Examples are given as specific illustrations of the claimed invention. It should be understood, however, that the invention is not limited to the specific details set forth in the Examples. All parts and percentages in the Examples as well as in the remainder of the specification are by weight unless otherwise specified.

Examples 1 - 7

These Examples illustrate the preparation of the enriched protein food product of the present invention. The following general procedure applies to each of these Examples.

One hundred twenty five (125) grams of the food product, one hundred (100) grams of water, and two point five (2.5) grams of finely divided calcium carbonate are mixed in a glass container. The container is closed with a cotton plug and the mixture is sterilized by heating in a steam autoclave. The mixture is then cooled to room temperature and innoculated with a Pleurotus ostreatus spawn culture. The innoculated mixture is then maintained in the presence of atmospheric air at 21°C. The specific details of each Example are given in Table I below.

TABLE I

EXAMPLES	1	2	3	4	5	6	7
Food Product	Corn	Rye	Oats	Rice	Wheat	Wheat 50% Corn 50% By Weight	White Acre Peas
Amount P.O. (grams)	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Initial Protein Content	11.3	14.3	14.0	8.0	15.5	12.8	22.5
Protein Content After 3 weeks	15.7	17.74	18.8	8.7	--	--	--
Protein Content After 4 Weeks	--	--	--	--	19.2	18.9	32.5
Protein Content After 5 Weeks	--	23.9	--	10.8	--	--	--

The protein content of each example was determined by the well known technique of nitrogen analysis.

The above examples illustrate that the present invention provides a process whereby protein content of a food product is substantially increased by innoculating the food product with mycelium of the genus Pleurotus. By means of this process, the ratio of protein to carbohydrate in cereal grains and other food products may be increased, thus alleviating to some degree the problems associated with the lack of protein in the diet of much of the world population.

Comparative Example

Example 2 was repeated except that Agaricus bisporus mycelium was used instead of Pleurotus ostreatus. Substantially no increase in protein content was measured.

The above-described Examples illustrate that when the process of the present invention is employed, there is produced a food product having an increased protein content. On the other hand, when the same process is carried out using Agaricus Bisporius rather than Pleurotus, there results substantially no protein increase.

The principles, preferred embodiments, and modes of operation of the present invention have been described in the foregoing specification. The invention which is intended to be protected herein, however, is not to be construed as limited to the particular forms disclosed, since these are to be regarded as illustrative rather than restrictive. Variations and changes may be made by those skilled in this art without departing from the spirit of the invention.

WHAT IS CLAIMED IS:

1. A process for increasing the protein content of a food product which contains carbohydrates comprising:

a) forming a mixture of said food product with water;

b) sterilizing said mixture in order to substantially eliminate the growth of substances which might compete with the growth of a spawn culture of the genus Pleurotus;

c) innoculating said mixture with a spawn culture of the genus Pleurotus;

d) maintaining the innoculated mixture in the presence of a mixture of nitrogen and oxygen at a temperature of from about 5°C to about 46°C so as to enable the mycelium of said spawn culture to reproduce; and

e) terminating the reproduction of said mycelium so as to form a food product which has an increased protein content.

2. The process for increasing the protein content of a food product as defined in claim 1 wherein said Pleurotus spawn culture comprises:

a member selected from the group consisting of Pleurotus ostreatus, Pleurotus ulmarius, Pleurotus sapidus, Pleurotus corninicopiae and Pleurotus florida.

3. The process for increasing the protein content of a food products as defined in claim 1 wherein:

the innoculated mixture formed in step (c), is maintained in the presence of air at a temperature of from about 10°C to about 46°C for from about 2 days to about 80 days.

4. The process for increasing the protein content of a food product as defined in claim 1 wherein the food product comprises:

a mixture of an agricultural waste product and a cereal grain.

5. The process for increasing the protein content of a food product as defined in claim 1 wherein the food product comprises:

at least one member selected from the group consisting of corn, rye, oats, rice, wheat and white acre peas.

6. The process for increasing the protein content of a food product as defined in claim 1 wherein:

from about 0.5% to about 20% by weight Pleurotus is added to the food product mixture based upon the total weight of the innoculated mixture.

7. A process for increasing the protein content of a food product which contains carbohydrates comprising:

a) forming a mixture of from about 20% to about 80% by weight food product and from about 20% to about 80% by weight water;

b) sterilizing said mixture in order to substantially eliminate the growth of substances which might compete with the growth of a spawn culture of the genus Pleurotus;

c) innoculating said mixture with from about 0.5% to about 20% by weight of at least one member selected from the group consisting of Pleurotus ostreatus, Pleurotus ulmarius, Pleurotus sapidus, Pleurotus corninicopiae, and Pleurotus florida;

d) maintaining the innoculated mixture in the presence of a mixture of nitrogen and oxygen at a temperature of from about 5°C to about 46°C so as to enable the mycelium of said spawn culture to reproduce; and

e) terminating the reproduction of said mycelium so as to form a food product which has a protein content which is about 30% to 65% by weight higher than it had been prior to said innoculation.

8. The process for increasing the protein content of a food product as defined in claim 7 wherein:

said mixture of food product and water comprises from about 30% to about 70% by weight food product and from about 30% to about 70% by weight water.

9. The process for increasing the protein content of a food product as defined in claim 8 wherein:

said mixture of nitrogen and oxygen comprises air.

10. The process for increasing the protein content of a food product as defined in claim 9 wherein:

said mixture is sterilized by pre-treating the food product with a chemical compound which removes substances which would grow and compete with Pleurotus.

11. The process for increasing the protein content of a food product as defined in claim 9 wherein:

the food product comprises a mixture of an agricultural waste product and a cereal grain.

12. A process for increasing the protein content of a food product which contains carbohydrates comprising:

a) forming a mixture of from about 40% to about 60% by weight food product and from about 40% to about 60% by weight water,

b) sterilizing said mixture in order to substantially eliminate the growth of substances which might compete with the growth of a spawn culture of the genus Pleurotus,

c) innoculating said mixture with from about 1% to about 5% by weight of at least one member selected from the group consisting of Pleurotus ostreatus, Pleurotus ulmarius, Pleurotus sapidus, Pleurotus corninicopiae, and Pleurotus florida,

d) maintaining the innoculated mixture in the presence of air at a temperature of from about 10°C to about 46°C, for from about 21 to about 35 days so as to enable the mycelium of said spawn culture to reproduce, and

e) terminating the reproduction of said mycelium so as to form a food product which has a protein content which is about 30% to 65% by weight higher than it had been prior to said innoculation.

13. The product produced by the process of claim 1.

14. The product produced by the process of claim 7.

15. The product produced by the process of claim 12.

INTERNATIONAL SEARCH REPORT WO 80/00400

International Application No PCT/US79/00670

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL. A23J 1/18
U.S. CL. 426/44

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	426/44,48,52,54,618,623,656; 47/1.1; 435/254

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

CHEMICAL ABSTRACT - PLEUROTUS 1956 - DATE DATA COMPUTER SEARCH - PLEUROTUS

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	US,A, 2,618,900, PUBLISHED 05 NOVEMBER 1952, SEE COLUMN 5, LINE 20 TO COLUMN 6, LINE 73, AND COLUMN 10, LINES 31-35, HUMFELD.	1-15
X	US,A, 2,648,163, PUBLISHED 11 AUGUST 1953, SEE EXAMPLE 1 AND COLUMN 4, LINES 3-11, SZUECS ET AL.	1-15
X	FR,A , 2,328,409, PUBLISHED 20 MAY 1977, SEE THE ABSTRACT.	1-15
X	US,A, 2,850,841, PUBLISHED 09 SEPTEMBER 1958, SEE EXAMPLE 4 AND COLUMN 3, LINES 59-64, SZUECS ET AL.	1-15
X	DE,A, 2,151,326, PUBLISHED 19 JULY 1973, SEE THE ABSTRACT.	1-15
X	CH,A , 527,554, PUBLISHED 31 OCTOBER 1972,SEE THE ABSTRACT.	1-15
X	DE,A, 2,750,009, PUBLISHED 18 MAY 1978, SEE THE ABSTRACT.	1-15
A	JP,A, 51-1252, PUBLISHED 07 JANUARY 1976.	1-15

* Special categories of cited documents: [15]

"A" document defining the general state of the art
"E" earlier document but published on or after the international filing date
"L" document cited for special reason other than those referred to in the other categories
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but on or after the priority date claimed
"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention
"X" document of particular relevance

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
12 DECEMBER 1979	17 JAN 1980
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	RAYMOND N. JONES

Form PCT/ISA/210 (second sheet) (October 1977)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	JP,A, 51-1253, PUBLISHED 07 JANUARY 1976.	1-15
A	JP,A, 51-1254, PUBLISHED 07 JANUARY 1976.	1-15
A	JP,A, 51-13663, PUBLISHED 03 FEBRUARY 1976.	1-15
A	JP,A, 51-13664, PUBLISHED 03 FEBRUARY 1976.	1-15
A	US,A, 2,505,811, PUBLISHED 02 MAY 1950, SZUECS.	1-15
A	US,A, 2,693,664, PUBLISHED 09 NOVEMBER 1954, SZUECS.	1-15
	SEE ATTACHED SHEET	

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

CONTINUED FROM FORM PCT/ISA/210 (SECOND SHEET) (OCTOBER 1977)
AND SUPPLEMENTAL SHEET (2)

III. DOCUMENTS CONSIDERED TO BE RELEVANT

CATEGORY*	CITATION OF DOCUMENT, WITH INDICATION, WHERE APPROPRIATE, OF THE RELEVANT PASSAGES	RELEVANT TO CLAIM NO.
A	US,A, 3,560,190, PUBLISHED 02 FEBRUARY 1971, HUGHES ET AL.	1-15
A	JP,A, 45-27768, PUBLISHED 10 SEPTEMBER 1970.	1-15
A	US,A, 3,286,399, PUBLISHED 22 NOVEMBER 1966, LANIECE.	1-15